

Microvascular and Macrovascular Reactivity Is Reduced in Subjects at Risk for Type 2 Diabetes

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Abnormalities in vascular reactivity in the micro- and macrocirculation are well established in type 2 diabetes. However, little is known about changes in vascular reactivity in those at risk for developing type 2 diabetes. To address this situation, the vascular reactivity in both the micro- and macrocirculation was studied in four age and sex comparable groups: 30 healthy normoglycemic subjects with no history of type 2 diabetes in a first-degree relative (controls), 39 healthy normoglycemic subjects with a history of type 2 diabetes in one or both parents (relatives), 32 subjects with impaired glucose tolerance (IGT), and 42 patients with type 2 diabetes without vascular complications (diabetes). Laser Doppler perfusion imaging was used to measure vasodilation in the forearm skin in response to iontophoresis of 1% acetylcholine chloride (Ach) (endothelium-dependent) and 1% sodium nitroprusside (SNP) (endothelium-independent), whereas high-resolution ultrasound images were used to measure brachial artery diameter changes during reactive hyperemia. Plasma concentrations of endothelin-1 (ET-1), vonWillebrand factor (vWF), soluble intercellular adhesion molecule (sICAM), and soluble vascular cell adhesion molecule (sVCAM) were also measured as indicators of endothelial cell activation. The vasodilatory responses to Ach, expressed as percent increase of blood flow over baseline, were reduced in relatives (98 ± 48 , mean \pm SD), IGT (94 ± 52), and diabetes (74 ± 45) compared with controls (126 ± 67) ($P < 0.001$ controls versus relatives, IGT, and diabetes). The responses to SNP were similarly reduced: controls (123 ± 46), relatives (85 ± 46), IGT (83 ± 48), and diabetes (65 ± 31) ($P < 0.001$ controls versus relatives, IGT, and diabetes) as were the responses in the brachial artery diameter during reactive hyperemia: controls (13.7 ± 6.1), relatives (10.5 ± 6.7), IGT (9.8 ± 4.5), and diabetes (8.4 ± 5.0) ($P < 0.01$ controls versus relatives, IGT, and dia-

betes). Women had greater responses than men in both the micro- and macrovascular circulatory tests, but a similar progressive reduction was observed in both sexes with increasing degrees of glucose intolerance. A significant inverse correlation was found between microvascular reactivity and systolic blood pressure, fasting plasma glucose, HDL cholesterol, fasting plasma insulin, and homeostasis model assessment (HOMA) values, an index of insulin resistance. BMI and diastolic blood pressure had a significant inverse correlation only with endothelium-dependent vasodilation. In the macrocirculation, systolic blood pressure, HbA_{1c}, HDL cholesterol, and HOMA had significant correlation with brachial artery diameter changes. Compared with control subjects, ET-1 was significantly higher in all groups, vWF was higher only in the diabetic group, sICAM levels were higher in the IGT and diabetic groups, while sVCAM concentrations were higher in the relatives and those with diabetes ($P < 0.05$). On stepwise multivariate analysis, age, sex, fasting plasma glucose, and BMI were the most important contributing factors to the variation of vascular reactivity. Addition of all clinical and biochemical measures explained only 32–37% of the variation in vascular reactivity. These results suggest that abnormalities in vascular reactivity and biochemical markers of endothelial cell activation are present early in individuals at risk of developing type 2 diabetes, even at a stage when normal glucose tolerance exists, and that factors in addition to insulin resistance may be operative. *Diabetes* 48:1856–1862, 1999

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Ach, acetylcholine chloride; ET-1, endothelin-1; HOMA, homeostasis model assessment; HRT, hormone replacement therapy; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; sICAM, soluble intercellular adhesion molecule; SNP, sodium nitroprusside; sVCAM, soluble vascular cell adhesion molecule; VSMC, vascular smooth muscle cell; vWF, von Willebrand factor.

Abnormalities in vascular reactivity in micro- and macrocirculation are well established in type 2 diabetes (1–3). Endothelial dysfunction is an important determinant of altered vascular reactivity and plays a major role in the genesis of micro- and macrovascular complications in diabetes (4,5). It is not known, however, how early these abnormalities can be detected in the prediabetic stage. Individuals with impaired glucose tolerance (IGT) and those with a parental history of diabetes have an increased risk of developing type 2 diabetes (6). There is little information regarding vascular reactivity in these groups. In microcirculation, reduced skin hyperemia to local heating in the feet of subjects with mild fasting hyperglycemia has been observed and associated with insulin resistance (7). In macrocirculation, endothelial dysfunction has been found to be present in obese insulin-resistant individuals with normal glucose tolerance (8); however, conflicting information exists as to whether insulin

resistance and endothelial dysfunction coexist in various study populations and whether there is a cause-effect relationship between them (9,10).

To test the hypothesis that abnormalities in vascular reactivity and endothelial function are present in both the micro- and macrocirculation in subjects at risk for developing type 2 diabetes, responses of the skin microcirculation to the iontophoresis of acetylcholine and sodium nitroprusside, as well as the brachial artery diameter changes during reactive hyperemia, were assessed in subjects with IGT and in normoglycemic individuals with a parental history of diabetes. For comparison, healthy control subjects and patients with established type 2 diabetes were also studied. Plasma concentrations of several markers of endothelial cell activation, including von Willebrand factor (vWF), endothelin-1 (ET-1), soluble vascular cell adhesion molecule (sVCAM), and soluble intercellular adhesion molecule (sICAM) were also measured in all groups.

RESEARCH DESIGN AND METHODS

Subjects. A total of 143 subjects aged 25–70 years were included in the study and divided in four groups. The control group consisted of 30 healthy subjects with a normal oral glucose tolerance test (OGTT) and no history of type 2 diabetes in any first-degree relative. The second group (relatives) consisted of 39 healthy individuals with a normal OGTT and a history of type 2 diabetes in one or both parents. The third group included 32 subjects with impaired glucose tolerance (IGT), and in the fourth group (diabetes) were 42 subjects who had an established diagnosis of type 2 diabetes. In the latter group, 18 patients were treated by diet alone, 8 were on a sulfonylurea (7 on glyburide and 1 on glipizide), 4 were on metformin, and 12 were on a combination therapy (10 on glyburide and metformin and 2 on glipizide and metformin). Diabetes and IGT were defined according to the recommendations of the American Diabetes Association (ADA) Expert Committee on the Classification and Diagnosis of Diabetes Mellitus (11).

There were nine premenopausal women, four postmenopausal women on hormone replacement therapy (HRT), and three postmenopausal women not receiving HRT in the control group. The corresponding numbers were 10, 4, and 6 in the relatives group; 8, 5, and 4 in the IGT group; and 7, 8, and 6 in the diabetic group. No statistically significant differences existed in any of the categories among the groups ($P = 0.81$). Two healthy premenopausal women and one from the IGT group were using oral contraceptive agents. All women were studied randomly in respect to their menstrual cycle.

To avoid confounding factors known to affect endothelial function and/or glucose metabolism, the following exclusion criteria were applied to subjects in all groups: subjects who smoked any amount of cigarettes during the previous 6 months and subjects with diagnosed cardiovascular disease (coronary artery disease, arrhythmia, heart failure), stroke or transient ischemic attack, peripheral vascular disease (symptoms of claudication and/or absence of peripheral pulses), chronic renal disease, severe dyslipidemia (triglycerides >600 mg/dl or cholesterol >300 mg/dl), or any other serious chronic disease requiring active treatment. Subjects were also excluded if they were on any of the following medications: any type of antihypertensives, lipid-lowering agents, glucocorticoids, antineoplastic agents, psychoactive agents, or bronchodilators. In addition, diabetic patients with proliferative retinopathy, peripheral somatic neuropathy, macroalbuminuria

(expressed as albumin/creatinine ratio >300 $\mu\text{g}/\text{mg}$), and/or on insulin or troglitazone were excluded from the study.

The protocol was approved by the ethics committee or institutional review board at each center, and all participants gave written informed consent. Volunteers for the study were recruited through local advertisement at the Joslin Diabetes Center and the Beth Israel Deaconess Medical Center in Boston. All subjects in the diabetes group were patients followed at these centers. Most of the people included in the groups at risk for diabetes were relatives of patients followed at the Joslin Diabetes Center. Some workers at these centers volunteered as healthy control subjects. All selected volunteers met the inclusion and exclusion criteria described above.

Methods. Subjects were studied after an overnight fast and a 24-h period of abstinence from alcohol and vigorous exercise. A standard 75-g OGTT was performed in those individuals without known history of diabetes to evaluate their glucose status. Eligible individuals were asked to come back for a second visit to the Joslin Clinical Research Center after an overnight fast of 12 h to perform the clinical and laboratory evaluations. Those taking sulfonylureas and/or metformin were asked not to take these medications for 12 h before the studies. A general physical examination was performed by a study physician. The diagnosis of proliferative retinopathy was made on the basis of clinical examination or a history of previous retinal laser treatment. The systolic and diastolic blood pressure readings were recorded to the nearest 2 mmHg as the mean of two measurements with the subjects seated. Subjects' weight, height, waist-to-hip ratio, and BMI were also obtained.

Blood samples were drawn from an antecubital vein with a 19-gauge needle without venous stasis. Plasma glucose, total serum cholesterol, and triglycerides were measured using the Synchron CX analyzer (Beckman Systems, Fullerton, CA), whereas HDL serum cholesterol was measured directly (Sigma, St. Louis, MO). LDL cholesterol was calculated from these results. The HbA_{1c} (normal range 4–6%) was determined in whole blood using ion-exchange high-performance liquid chromatography. Plasma insulin was measured using the radioimmunoassay method. ET-1 (American Research Products, Belmont, Massachusetts), von Willebrand Factor (vWF) (Asserachrom, American Bioproducts, Parsippany, New Jersey), sVCAM, and sICAM (R&D Systems Minneapolis, Minnesota) were measured in plasma in duplicate using the enzyme-linked immunosorbent assay method. Insulin resistance was assessed by using the homeostasis model assessment (HOMA), which is based on a mathematical correlation of fasting plasma glucose and insulin levels (12).

Vascular reactivity tests. All vascular reactivity measurements and clinical evaluations were performed on the same morning while the subjects were still fasting. The investigators who performed the macrocirculation (R.S.) and microcirculation (P.S.) measurements were blinded to the medical history of the subjects. These studies were carried out in a temperature-controlled room (24–26°C) and after a 30-min acclimatization period.

The vascular reactivity of the forearm skin microcirculation was evaluated by laser Doppler perfusion imaging measurements before and after the iontophoresis of acetylcholine chloride (ACh) (endothelium-dependent vasodilation) and sodium nitroprusside (SNP) (endothelium-independent vasodilation) (1). The reproducibility of the technique has been previously reported by our group (13). The coefficient of variation of the baseline measurement was 14.1%, and during maximal hyperemic response after the iontophoresis, 13.7%.

To assess the vascular reactivity in the macrocirculation, the flow mediated brachial artery dilation was measured at rest and during reactive hyperemia by using a high-resolution ultrasound with a 10.0-MHz linear array transducer and an HDI Ultramark 9 system (Advanced Technology Laboratories, Bothel, Washington). Reactive hyperemia is produced by inflating a pneumatic tourniquet proximally to the brachial artery to 50 mmHg above the systolic pressure for 5 min and then deflating it. This protocol is described in detail elsewhere (14). We have found

TABLE 1
Clinical characteristics of studied groups

	Controls (C)	Relatives (R)	IGT	Diabetes (D)
<i>n</i>	30	39	32	42
Age (years)	48 ± 9	49 ± 10	50 ± 10	53 ± 9
Sex (M/F)	14/16	19/20	15/17	21/21
Systolic blood pressure (mmHg)*	114 ± 11	122 ± 8	123 ± 11	124 ± 9
Diastolic blood pressure (mmHg)†	73 ± 8	79 ± 7	79 ± 8	80 ± 6
BMI‡	26.4 ± 3.7	28.0 ± 4.6	32.6 ± 7.6	32.2 ± 6.3
Waist-to-hip ratio§	0.84 ± 0.08	0.87 ± 0.09	0.91 ± 0.10	0.92 ± 0.08

Data are means ± SD. *C vs. R, IGT, and D, $P < 0.001$; †C vs. R, IGT, and D, $P < 0.01$; ‡C and R vs. IGT and D, $P < 0.001$; §C vs. IGT and D, $P < 0.001$.

TABLE 2
Results of biochemical measurements

	Controls (C)	Relatives (R)	IGT	Diabetes (D)
Fasting glucose (mg/dl)*	92 ± 7	95 ± 7	105 ± 10	170 ± 58
Fasting insulin (µU/ml)†	5 (2–47)	5 (3–25)	11 (4–45)	13.5 (3–95)
Relative insulin resistance (HOMA model)‡	20 (7–188)	23 (10–140)	83 (26–320)	118 (49–187)
2-h post OGTT glucose§	104 ± 23	108 ± 20	168 ± 20	—
HbA _{1c} (%)	5.5 ± 0.5	5.7 ± 0.4	5.7 ± 0.5	8.0 ± 1.6
Triglycerides (mg/dl)¶	79 (37–209)	79.5 (33–288)	125 (31–599)	171 (54–507)
Total cholesterol (mg/dl)#	194 ± 34	198 ± 35	219 ± 37	210 ± 38
HDL (mg/dl)**	54 ± 15	49 ± 17	48 ± 13	42 ± 10
LDL (mg/dl)	120 ± 31	128 ± 36	143 ± 33	134 ± 36

Data are means ± SD or medians (range). *C, R, and IGT vs. D, *P* < 0.001; †C and R vs. IGT and D, *P* < 0.001; ‡C and R vs. IGT vs. D, *P* < 0.001; §C and R vs. IGT, *P* < 0.001; ||C, R, and IGT vs. D, *P* < 0.001; ¶C and R vs. IGT and D, *P* < 0.001; #C and R vs. IGT, *P* < 0.05; **C and R vs. D, *P* < 0.05.

a very strong correlation between measurements obtained when placing the tourniquet proximally and distally to the brachial artery in healthy subjects (15). This method has also shown a very close association with the endothelial function at the level of the coronary arteries (16).

Data analysis. The Minitab statistical package (Minitab, State College, Pennsylvania) for personal computers was used for the statistical analysis. For parametrically distributed data, the analysis of variance test was used, followed by the Fisher's test to identify differences among the various groups. For nonparametrically distributed data, the Kruskal-Wallis test was used. Correlation between variables was tested using both univariate and multivariate analyses (Pearson correlation analysis and multiple stepwise regression analysis).

RESULTS

The results of the anthropometric measurements and clinical examination are shown in Table 1. All groups had comparable age and sex. The mean duration of diabetes in the fourth group was 4.3 years (range 1–20). Microalbuminuria (urinary albumin-to-creatinine ratio between 30 and 300 µg/mg) was present in five men and six women in this group.

The results of biochemical tests are shown in Table 2. Compared with controls, fasting plasma triglycerides and insulin concentrations were higher in the IGT and diabetes groups, the total cholesterol was higher in the IGT group, and the HDL cholesterol was lower in the diabetic group. The index of insulin resistance (HOMA) was higher in the IGT group and highest in the diabetic group. There were no statistically significant differences between controls and relatives regarding BMI, waist-to-hip ratio, triglycerides, HDL cholesterol, LDL cholesterol, insulin, and HOMA values (Tables 1 and 2). Table 3 shows the plasma levels of the markers of endothelial activation. ET-1 levels were significantly higher in subjects with diabetic relatives, as well as in the IGT and dia-

betes groups when compared with the controls. The vWF was higher in subjects with diabetes compared with controls and relatives. sICAM levels were higher in the IGT and diabetic group when compared with controls and in the diabetic group when compared with the relatives. Finally, sVCAM levels were higher in the relatives and diabetic patients when compared with control subjects.

Resting skin temperature and blood flow in the forearm skin microcirculation were similar in all four groups (Table 4). The percent increase of blood flow over baseline after the iontophoresis of Ach was reduced in all groups compared with controls. A significant difference was also observed between the relatives and diabetic subjects (Fig. 1). The same pattern of results was observed during the iontophoresis of SNP. A very close correlation was found between baseline blood flow measurements before the iontophoresis of Ach and those before the iontophoresis of SNP (*r* = 0.92, *P* < 0.0001), a testament of the reliability of the laser Doppler scanner imaging technique.

In the macrocirculation, there were no differences among the four groups in the diameter of the brachial artery under basal conditions (Table 4). However, the percent increase in the brachial artery diameter during reactive hyperemia (endothelium-dependent vasodilation) was significantly reduced in all groups compared with controls. The resting heart rate was lower in the controls and relatives compared with the IGT and diabetic subjects, but no differences were found in the heart rate during the hyperemic response. No differences were present in the average and peak blood flow velocities at the brachial artery before and during the hyperemic response in the four groups.

TABLE 3
Results of vWF, ET-1, sICAM, and sVCAM plasma levels

	Controls (C)	Relatives (R)	IGT	Diabetes (D)
vWF (%)*	110 ± 49	103 ± 41	121 ± 45	135 ± 51
ET-1 (pg/ml)†	3.8 (1.4–12.3)	5.3 (1.2–33.4)	6.8 (1.3–43.1)	5.4 (1.5–39.1)
sICAM (ng/ml)‡	222 ± 57	251 ± 89	264 ± 56	301 ± 106
sVCAM (ng/ml)§	661 ± 176	747 ± 171	759 ± 254	831 ± 257

Data are means ± SD or medians (range). *C and R vs. D, *P* < 0.05; †C vs. R, IGT, and D, *P* < 0.05; ‡C vs. IGT, and D and R vs. D, *P* < 0.01; §C vs. R and D, *P* < 0.05.

TABLE 4
Results of micro- and macrovascular reactivity

	Controls (C)	Relatives (R)	IGT	Diabetes (D)
Skin temperature (°C)	31.0 ± 1.0	30.9 ± 1.0	30.6 ± 1.2	30.7 ± 0.9
Baseline blood flow before the iontophoresis of acetylcholine (V)	0.78 ± 0.21	0.69 ± 0.15	0.71 ± 0.18	0.71 ± 0.14
Blood flow after the iontophoresis of acetylcholine (V)*	1.72 ± 0.56	1.35 ± 0.37	1.32 ± 0.31	1.22 ± 0.37
Percent increase over baseline†	126 ± 67	98 ± 48	94 ± 52	74 ± 45
Baseline blood flow before the iontophoresis of SNP (V)	0.81 ± 0.21	0.72 ± 0.17	0.73 ± 0.20	0.73 ± 0.15
Blood flow after the iontophoresis of SNP (V)‡	1.79 ± 0.56	1.33 ± 0.44	1.31 ± 0.40	1.20 ± 0.33
Percent increase over baseline§	123 ± 46	85 ± 46	83 ± 48	65 ± 31
Resting brachial artery diameter (mm)	3.6 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
Percent increase over baseline after reactive hyperemia	13.7 ± 6.1	10.5 ± 6.7	9.8 ± 4.5	8.4 ± 5.0
Resting heart rate (beat/min)¶	63 ± 12	64 ± 11	72 ± 12	76 ± 14
Heart rate after reactive hyperemia (beats/min)#	65 ± 12	66 ± 16	74 ± 11	84 ± 18
Resting blood velocity (m/min)	16 (6–57)	21 (8–103)	18 (9–50)	18 (9–47)
Average blood velocity after reactive hyperemia (m/min)	133 (44–188)	122 (38–163)	133 (88–199)	115 (52–192)
Peak resting blood velocity (m/min)	90 (58–132)	92 (56–174)	92 (57–147)	93 (59–185)
Peak blood velocity after reactive hyperemia (m/min)	221 (112–298)	216 (95–289)	229 (106–312)	197 (84–296)

Data are means ± SD or medians (range). *C vs. R, IGT, and D, $P < 0.001$; †C vs. R, IGT, and D, and R vs. D, $P < 0.001$; ‡C vs. R, IGT, and D, $P < 0.001$; §C vs. R, IGT, and D, and R vs. D, $P < 0.001$; ||C vs. R, IGT, and D, $P < 0.01$; ¶C and R vs. IGT and D, $P < 0.01$; #C and R vs. D, $P < 0.01$.

No statistically significant differences in the vascular reactivity in the micro- and macrocirculation were seen when the results in the group of people with diabetes were compared between those with and without microalbuminuria and between those controlled on diet and those on sulfonylurea, metformin, or combination therapy (data not shown).

When the analysis was made by sex, it was found that the vasodilatory response in both the micro- and macrocirculation was significantly higher in women compared with men, independent of group (Fig. 2). The HDL cholesterol was lower in men compared with women in all groups (controls 45 mg/dl ± 11 vs. 61 ± 15, relatives 43 ± 10 vs. 55 ± 20, IGT 43 ± 6 vs. 52 ± 17, diabetes 37 ± 8 vs. 47 ± 10, $P < 0.05$ in all groups) while LDL cholesterol was higher in control males compared with control females (135 ± 34 vs. 108 ± 21, $P < 0.05$) and triglycerides were higher in men with IGT compared with women of the same group (170 ± 92 vs. 114 ± 43, $P < 0.05$). No other statistically significant clinical or biochemical differences were observed between men and women (data not shown).

When all subjects were considered as one group, univariate linear regression analysis showed significant correlations among all vascular reactivity measurements. Thus, the response to Ach correlated with the response to SNP ($r = 0.61$, $P < 0.001$) and the percentage change in brachial artery diameter during reactive hyperemia ($r = 0.36$, $P < 0.001$). The correlation between the response to SNP and change in brachial artery diameter was also significant ($r = 0.41$, $P < 0.001$).

Table 5 shows the relationship between vascular reactivity and clinical, metabolic, and biochemical parameters. On stepwise multivariate analysis, sex, fasting blood glucose, BMI, and age contributed to the variation in the response to

Ach by 20, 7, 3, and 4% respectively. The addition of all the other measurements in the model increased the prediction to 37%. The variation of the response to SNP could be predicted by sex (20%), fasting blood glucose (7%), and sVCAM levels (3%). Addition of all the other measurements increased the prediction of variation to 34%. The brachial artery diameter change in response to reactive hyperemia could be predicted by sex (13%), age (7%), and sVCAM levels (3%), while the addition of the rest of the measurements increased the prediction of variation to 32%. Similar results were found when each of the study groups was analyzed separately (data not shown).

DISCUSSION

The main finding of this study is that vascular reactivity in both the micro- and macrocirculation is reduced in subjects with IGT and in normoglycemic individuals with a parental history of diabetes when compared with healthy control subjects. Our results of decreased vascular reactivity in the microcirculation of the skin in people with type 2 diabetes are consistent with a previous report (1). In this study, we are extending the findings of altered vascular reactivity in type 2 diabetes to those at risk for developing type 2 diabetes. Until now, there has been little prior information regarding skin microcirculatory responses in individuals with impaired glucose tolerance (7) and no information in first-degree relatives of patients with type 2 diabetes.

In our study, we assessed endothelium-dependent and endothelium-independent responses in skin microcirculation. The responses to both Ach and SNP were reduced in all study groups compared with controls. It is known that Ach stimulates nitric oxide (NO) production in endothelial cells

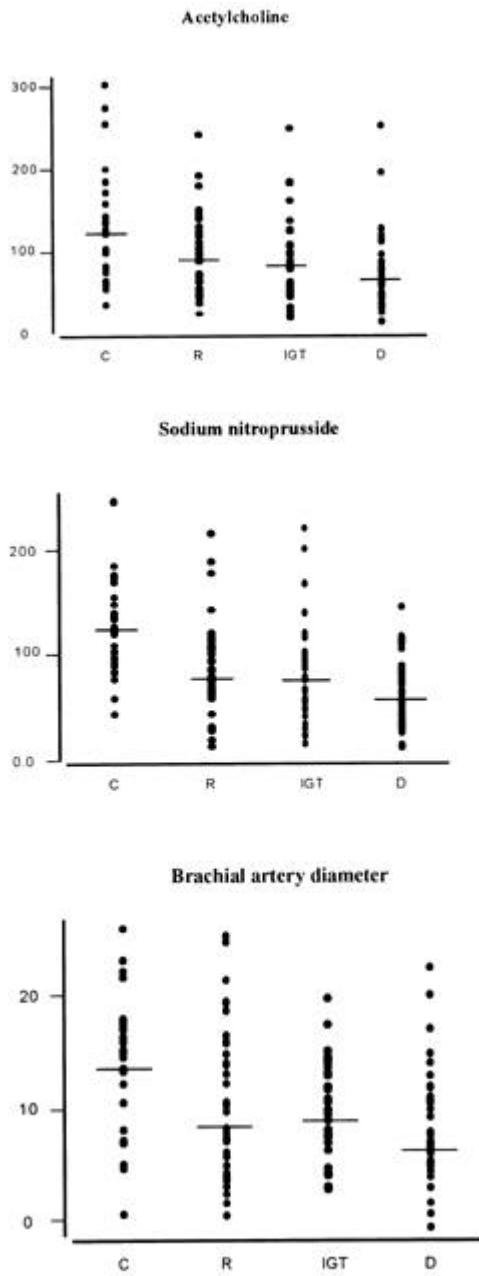


FIG. 1. The response to the iontophoresis of acetylcholine (endothelium-dependent vasodilation in the microcirculation), SNP (endothelium-independent vasodilation in the microcirculation), and the brachial artery diameter change in response to reactive hyperemia (endothelium-dependent vasodilation in the macrocirculation) were reduced in first-degree relatives of type 2 diabetic patients (group R), subjects with impaired glucose tolerance (IGT), and type 2 diabetic patients (group D) when compared with healthy controls (group C). In addition, the acetylcholine and SNP response in the relatives was higher when compared with diabetic patients. Results are presented as percent increase over baseline.

(endothelium-dependent vasodilation). SNP on the other hand acts as an NO donor to vascular smooth muscle cells (VSMCs) (endothelium independent vasodilation). Our study cannot discriminate whether the abnormal responses are due to a decreased production of NO, an increased destruction or inactivation of NO, or a decreased VSMC responsiveness to it. VSMCs possess an insulin-responsive NO synthase that may play a role in this cascade of events (17). Multiple abnormal-

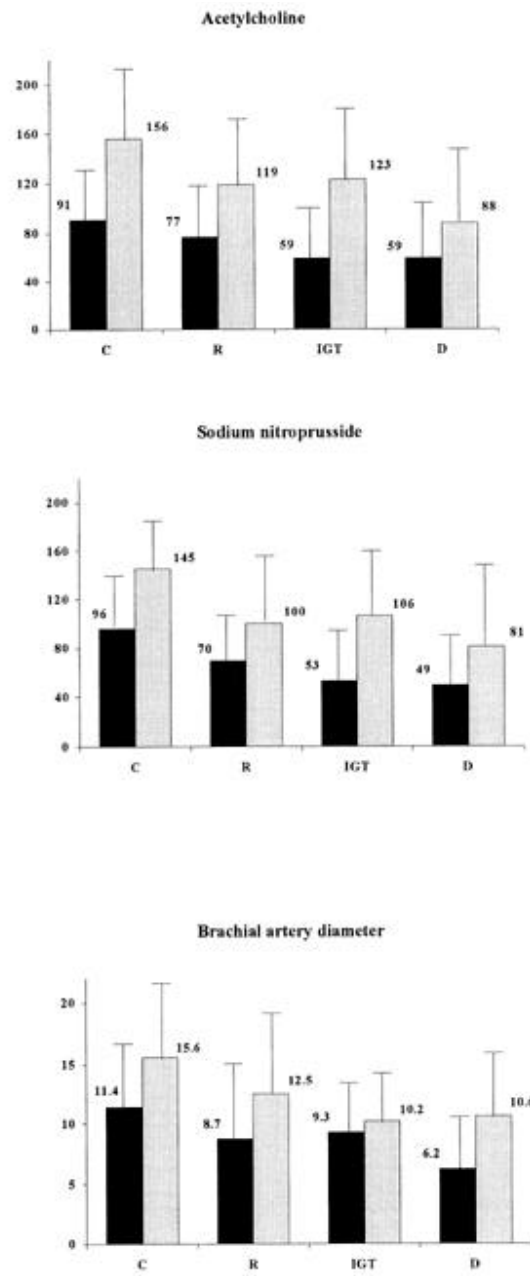


FIG. 2. The response to the iontophoresis of acetylcholine (endothelium-dependent vasodilation in the microcirculation), SNP (endothelium-independent vasodilation in the microcirculation), and the brachial artery diameter change in response to reactive hyperemia (endothelium-dependent vasodilation in the macrocirculation) in healthy men (■) and women (□) (group C), first-degree relatives of type 2 diabetic patients (group R), subjects with impaired glucose tolerance (IGT), and type 2 diabetic patients (group D). A stepwise reduction was observed in both men and women when analyzed separately, but women gave responses that were higher compared with those of men in all groups ($P < 0.001$). Results are presented as percent increase over baseline and expressed as means \pm SD.

ities may be operative, and some of them have been associated with diabetes, including smooth muscle dysfunction (18,19).

The brachial artery diameter change during reactive hyperemia (endothelium-dependent vasodilation) was also reduced in subjects with IGT and in those with a parental history of diabetes. Endothelial dysfunction has been widely observed in clinical studies in subjects with type 2 diabetes (2,3). Most

TABLE 5

Correlation of the response (percent increase over baseline) to acetylcholine, SNP, and brachial artery diameter in response to reactive hyperemia to various relevant parameters

	Response to acetylcholine	Response to nitroprusside	Brachial artery diameter
Age	-0.28 (0.001)	-0.23 (0.006)	-0.30 (0.000)
Systolic blood pressure	-0.28 (0.001)	-0.283 (0.001)	-0.27 (0.002)
Diastolic blood pressure	-0.25 (0.003)	-0.13 (0.125)	-0.08 (0.345)
BMI	-0.18 (0.03)	-0.05 (0.554)	-0.07 (0.439)
Fasting glucose	-0.25 (0.003)	-0.25 (0.003)	-0.12 (0.168)
2-h post OGTT glucose	-0.19 (0.054)	-0.17 (0.1)	-0.27 (0.008)
HbA _{1c}	-0.26 (0.002)	-0.25 (0.003)	-0.17 (0.049)
Triglycerides	-0.29 (0.000)	-0.21 (0.012)	-0.13 (0.132)
HDL	0.31 (0.000)	0.34 (0.000)	0.26 (0.002)
LDL	-0.05 (0.560)	-0.05 (0.592)	-0.06 (0.521)
Total cholesterol	-0.06 (0.496)	-0.01 (0.944)	-0.05 (0.536)
sICAM	-0.11 (0.196)	-0.23 (0.006)	-0.08 (0.343)
sVCAM	-0.04 (0.627)	-0.29 (0.001)	-0.26 (0.002)
vWF	-0.19 (0.028)	-0.09 (0.279)	0.13 (0.136)
Endothelin	0.03 (0.746)	0.01 (0.904)	-0.06 (0.550)
Fasting insulin	-0.25 (0.004)	-0.24 (0.005)	-0.148 (0.086)
Insulin resistance	-0.28 (0.001)	-0.26 (0.002)	-0.13 (0.0142)

Data are r (P).

of the metabolic abnormalities frequently present in diabetic patients have been independently associated with endothelial dysfunction, including hyperglycemia (20), obesity (8), hypercholesterolemia (14, 21), low HDL cholesterol levels (22), and hypertension (23). However, there is some controversy as to whether endothelial dysfunction and insulin resistance consistently coexist and whether there is a cause-effect relationship between them (8–10,24).

Some differences in clinical and metabolic characteristics were observed among our study groups. Those individuals with IGT exhibited some features of insulin resistance widely accepted to be present in this group (25). The group of first-degree relatives also had slightly higher blood pressure levels compared with controls, although still in the normal range. Even though statistically significant differences in most metabolic variables in this group of relatives compared with controls were not found, they had slightly higher BMI and waist-to-hip ratio, consistent with the features of insulin resistance that have often been described in this population (26,27). Statistically significant inverse correlations were found between vascular reactivity and age, systolic and diastolic blood pressure, BMI, fasting plasma glucose, fasting insulin levels, and index of insulin resistance (HOMA). A direct correlation was found with HDL cholesterol. However, during stepwise regression analysis, only age, sex, fasting plasma glucose, and BMI were identified as significant contributing factors to vascular reactivity. Despite the significant contribution of these factors to vascular reactivity, only 32–37% of the variation in the responses could be explained by the addition of all measured variables in our study. Recent literature suggests that genetic factors operating at times when no obvious metabolic abnormalities are seen may be involved in vascular dysfunction (28). Most likely, a combination of genetic factors and acquired metabolic abnormalities interplay in determining vascular health and disease. More studies are needed to understand the sequence of events leading to abnormal vascular function in subjects at risk for type 2 diabetes.

Another important finding in our study was the sex difference in vascular reactivity. It has been previously shown that nondiabetic women have better endothelial function measurements in the macrocirculation (29). Our results confirm this observation and show similar findings in the microcirculation. There is some information suggesting a beneficial effect of estrogen replacement therapy on both micro- and macrocirculation in healthy women (30,31). A possible limitation in the present study is that women were evaluated randomly in regards to their menstrual period. However, the proportion of pre- and postmenopausal women with and without HRT was not statistically different among groups. Thus, women have an overall better vascular reactivity than men, but no valid conclusion can be drawn about the differences according to menopausal status and HRT due to the small number of subjects in each subgroup.

Cellular adhesion molecules are expressed on endothelial cells in response to inflammation and facilitate the adhesion of circulating leukocytes to their surface, an early step in their transendothelial migration and the development of atherosclerosis. Increased sICAM-1 levels in healthy individuals are associated with higher risk of future myocardial infarction (32), while both sICAM-1 and sVCAM-1 have been reported to be higher in subjects with IGT and diabetes in some but not all published studies (33,34). Release of vWF is another response of the activated endothelium and is associated with the development of long-term diabetes complications (35). Finally, ET-1, a potent vasoconstrictor secreted by the endothelium, plays a role in the regulation of blood pressure and has been found to be elevated in patients with essential hypertension (36). High levels of ET-1 and failure of ET-1 to produce vasoconstriction have been reported in patients with type 2 diabetes (37,38).

In the present study, subjects with type 2 diabetes had significantly higher levels of sVCAM, sICAM, vWF, and ET-1 compared with controls. Subjects with IGT had higher levels of sICAM and ET-1, whereas the first-degree relatives exhibited higher plasma concentrations of sVCAM and ET-1.

In conclusion, in the present study, we have shown that vascular reactivity in the skin microcirculation and in the brachial artery is impaired in individuals with IGT and in normoglycemic subjects with a parental history of type 2 diabetes. These two groups also had increased levels of ET-1 and cellular adhesion molecules consistent with endothelial activation. Women had better vascular reactivity than men regardless of glucose homeostasis stage. The association of abnormalities in vascular reactivity in different territories of the micro- and macrocirculation and the development of diabetes-associated vascular complications is still unclear and deserves further investigation. However, the present study demonstrates that abnormalities in vascular function are present in subjects at risk for developing type 2 diabetes, even at a stage of normal glucose tolerance. Understanding how insulin resistance and the various concomitant metabolic abnormalities interplay with genetic factors in producing vascular dysfunction may lead to the generation of strategies that could target early abnormalities in the development of vascular complications in type 2 diabetes.

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